

comprising at least a first and a second subpopulation, said microspheres comprising a bioactive agent, and wherein said sites can have only a single microsphere.

25. (New) A composition comprising:

a) a substrate with a patterned surface comprising discrete sites, said substrate comprising discrete sites at a density of at least 100 discrete sites per 1 mm² ; and

b) a population of microspheres, randomly distributed on said sites, wherein each microsphere comprises a bioactive agent; and
wherein said sites can have only a single microsphere.

26.(New) A composition according to claim 24 or 25 wherein said substrate is a fiber optic bundle.

27. (New) A composition according to claim 24 or 25 wherein said substrate is selected from the group consisting of glass and plastic.

28. (New) A composition according to claim 24 wherein said population of microspheres comprises at least a first and a second subpopulation, wherein the microspheres of said first subpopulation of microspheres are a different size than the microspheres of said second subpopulation.

29. (New) A composition according to claim 24 or 25 wherein said bioactive agent comprises a protein.

30. (New) A composition according to claim 29 wherein said protein is selected from the group consisting of enzymes and antibodies.

31. (New) A composition according to claim 24 or 25 wherein said bioactive agent is a nucleic acid.

32. (New) A composition according to claim 25 wherein said population of microspheres comprises at least a first and a second subpopulation, wherein the microspheres of said first subpopulation of microspheres are a different size than the microspheres of said second subpopulation.

33. (New) A composition according to claim 24 or 28, wherein said first and said second subpopulations comprise a first and a second bioactive agent, respectively.

34. (New) The composition according to claim 32, wherein said first and second subpopulations further comprise a first and a second optical signature, respectively.

35. (New) A composition according to claim 33 wherein said at least one of said optical signatures comprises at least one chromophore.

36. (New) A composition according to claim 33 wherein said at least one of said optical signatures comprises at least one fluorescent dye.

37. (New) A composition according to claim 35 wherein said fluorescent dye is entrapped within said microspheres.

38. (New) A composition according to claim 35 wherein said fluorescent dye is attached to said microspheres.

39. (New) A composition according to claim 33 wherein said optical signature comprises at least two fluorescent dyes.

40. (New) A composition according to claim 32 wherein said bioactive agent comprises a protein.

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41. (New) A composition according to claim 32 wherein said protein is selected from the group consisting of enzymes and antibodies.

42. (New) A composition according to claim 32 wherein said bioactive agent is a nucleic acid.

43. (New) A composition according to claim 24 or 25 wherein said bead is covalently associated with the well.

44. (New) A composition according to claim 24 or 25 wherein said bead is non-covalently associated with the well.

45. (New) A method of determining the presence of at least a first and second target analyte in a sample comprising:

a) contacting said sample with a composition comprising:

i) a substrate with a patterned surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation, wherein said first subpopulation comprises a first bioactive agent and said second subpopulation comprises a second bioactive agent, wherein said microspheres are randomly distributed on said surface such that said discrete sites contain only one microsphere; and

b) determining the presence of said first and second target analyte.

46. (New) A method according to claim 39 wherein said substrate is a optical fiber bundle and said microspheres are located within wells at a first terminal end of said bundle.

47. (New) A method according to claim 39 further comprising identifying the location of said first and second bioactive agent on said substrate.

48. (New) The method according to claim 39, wherein said discrete sites are wells.

49. (New) The method according to claim 39, wherein said substrate is selected from the group consisting of glass and plastic.

50. (New) A method of making a composition comprising:

- a) providing a patterned surface comprising individual sites on a substrate;
- b) randomly distributing microspheres on said surface such that said individual sites contain microspheres, wherein said sites can have only a single microsphere, and wherein said microspheres comprise at least a first and a second subpopulation comprising:
 - i) a first and second bioactive agent, respectively; and
 - ii) a first and second optical signature, respectively;
- c) detecting said first and second optical signatures while said microspheres are distributed on said surface; and
- d) correlating the location of at least one individual site on the array with the bioactive agent at that particular site.

51. (New) A method according to claim 44, wherein said distributing comprises serially adding said subpopulations to said sites.